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STUDIES ON SEX-DETERMINATION IN AMPHIBIANS. II.

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It is still an open question whether external factors have any influence on the determination of sex. Much of the experimental work that has seemed to show that sex can be influenced by nutrition, temperature, etc., has either been carried out with too small a number of individuals or the methods used in the experiments have been too crude to give results that could be considered as decisive. It is seemingly quite within the power of the modern investigator to determine definitely whether sex can be influenced by external factors; and careful work, such as that which has been done by Cuénot and Hertwig, should settle this question for all time. If it is found that external factors do influence sex then the current chromosome-sex theory will have to be abandoned or considerably modified; if, on the other hand, it is shown convincingly that external factors have no influence on sex-determination, then the way is cleared for the theory that best explains the facts known to us and offers the most favorable hypothesis for future investigations along this line.

Several years ago I started a series of experiments on the eggs of the common American toad, *Bufo lentiginosus*, in order to study the problem of sex-determination in this amphibian which seems to furnish peculiarly favorable material for an investigation of this kind. In the first experiments that were made the influence of nutrition on the determination of sex was investigated. The results of this work, as shown in a previous paper (King, '07), seem to indicate that neither the quantity nor the quality of the food given the larvæ has any influence on sex. The present paper records the results of the second series of experiments that

was made to study the determination of sex in *Bufo*. The work was carried on in the vivarium of the University of Pennsylvania where the splendid facilities at my command permitted the use of relatively large numbers of individuals in each experiment and the rearing of them under exceptionally favorable conditions.

In all of the experiments in this series the tadpoles were kept in large glass aquaria or in cement tanks which were supplied with running water. Each tank contained a sloping bank of sand and gravel and also a quantity of water plants. With the exception of the one experiment in which the influence of starvation on the determination of sex was being studied, all of the tadpoles used in these investigations received similar food, viz., water plants and minute organisms in abundance and occasionally fine pieces of cooked meat or cereal. According to my observations tadpoles of *Bufo* invariably prefer an animal to a vegetable diet and they readily devour the dead bodies of their companions. To this latter fact can be attributed the loss of many individuals during the course of these experiments. Since there is no apparent relation between mortality and sex among tadpoles reared under artificial conditions, as shown by the investigations of Pflüger ('82), and also by my former work, the individuals in which sex was not ascertained are disregarded in considering the results of these experiments, although the total number of individuals with which each experiment started is given in most cases. The methods used in ascertaining the sex of the individuals were similar to those previously employed (King, '07). Whenever possible the bodies of tadpoles that died were preserved and the sex ascertained by means of sections. As there is considerable individual variation in the size and also in the development of the gonads, even after the metamorphosis of the toads, it was necessary to section the genital organs of nearly one fourth of the total number of individuals in order to ascertain their sex.

I. Experiments to Determine the Sex Ratio of the Individuals that Develop from the Eggs of the Right Ovary and of those from the Left Ovary.

If sex is determined in the ovary, as many investigators believe, it is possible that the individuals developing from the eggs

of one ovary may show a very different sex ratio from that of the individuals produced from the eggs of the other ovary; or it may be that the eggs of one ovary produce only females and those of the other ovary only males. The following experiments were made to determine this point for *Bufo*. On April 26, 1907, a female which had just begun to deposit her eggs was killed by pithing. The eggs in the right uterus were separated from those in the left uterus and each set was artifically fertilized, sperm from the same male being used for both lots. Six hundred eggs from each ovary were taken for the purposes of the experiment and placed in tanks of equal size. During the course of their development all of the tadpoles were subjected to similar conditions of temperature and they received similar food. The sex of the first 300 individuals in each set to undergo metamorphosis was ascertained with the result shown in the following table:

TABLE I.

	Total Sex Ascertained.	Males.	Females.	Per Cent. of Females.
Right Ovary.....	300	131	169	56.33
Left Ovary.....	300	128	172	57.33
Total.....	600	259	341	56.83

As shown in the above summary both lots of individuals gave approximately the same sex ratio; 56.33 per cent. of the individuals from the right ovary and 57.33 per cent. of those from the left ovary being females. This proportion of females is somewhat higher than that obtained in the majority of the experiments and seems to indicate that there is considerable normal variation in the sex ratio of lots of eggs laid by different females.

The above experiment was repeated with the eggs of another toad in the spring of 1908. Owing to an accident, however, all of the tadpoles developing from the eggs of the left ovary were killed and in only 140 of the individuals that had developed from the eggs of the right ovary was sex ascertained. Of this number 64 individuals were males and 76, or 54.2 per cent., were females.

From the results obtained in these two experiments it is evident that the sex ratio of the individuals produced from the eggs of the right ovary is not materially different from that of the indi-

viduals which come from the eggs of the left ovary. Whatever the factors may be which normally determine sex in *Bufo* they appear to have a similar action on the eggs of both ovaries.

II. The Influence of Starvation on Sex-Determination in Bufo.

During the course of my former experiments an attempt was made to ascertain whether a scarcity of food tends to produce a relatively greater number of males, as several investigators have maintained. The mortality among the young tadpoles was so great, however, that the experiment was abandoned for the time. In the spring of 1907 this experiment was repeated on a much larger scale. Eight hundred eggs which had been laid and normally fertilized in the laboratory on the morning of March 30, were placed in a large tank containing only water and clean sand. No food of any kind was given the tadpoles at first and, as might be expected, they began dying in great numbers about two weeks after hatching. Until the beginning of May the only food that the larvæ obtained was the dead bodies of their companions which were devoured before they could be removed. The tadpoles that lived developed very slowly, and in order to prevent all of them from dying they were given a small amount of meat on the second of May and once a week thereafter. Of the 800 individuals with which the experiment started only 59 lived until their sex could be ascertained. Of this number 24 were males and 35, or 59.32 per cent., were females. This number of individuals is, of course, too small to be of much value for statistical purposes, yet the results of the experiment, as far as they go, seem to indicate that scarcity of food has no influence whatever on sex-determination in *Bufo*. Since the only food that the tadpoles received was animal food it might be inferred, from the rather large proportion of females that were obtained, that nitrogenous food favors the production of females, as Yung ('85) has maintained. The excess of females does not seem to me sufficiently large, however, to warrant such a conclusion. Moreover my previous experiments showed that in a total of 464 individuals that received an exclusive meat diet only 246, or 53.01 per cent., were females, thus making it highly improbable that nitrogenous food has any influence on the determination of sex in *Bufo*.

III. The Influence of the Ripeness of the Egg at the Time of Fertilization on Sex-Determination in Bufo.

For several years past Hertwig ('05–'07) has been investigating the problem of sex-determination, principally in various species of *Rana*, and he has already published three contributions to the literature on this subject. In his first paper, which appeared in 1905, Hertwig ('05) suggests that the sexual character of an egg changes during the course of its development in the ovary. In the early stages of its ripening the egg tends to produce a male; in the middle phase of its ripening its tendency is towards the production of a female; and in later phases, when the egg is overripe, it again shows a tendency to develop into a male. This change in the sexual character of the egg Hertwig ascribes in his second paper ('06) to "einem verschiedenen Wechselverhältnis ihrer Hauptbestandteile, der Kernsubstanz und des Protoplasma. . . . Eier, welche relativ ärmer an Kernsubstanz sind, Weibchen liefern, chromatinreichere dagegen Männchen." In his latest communication Hertwig ('07) lays little emphasis on the second part of his theory, but he seems to consider that the results of his experiments warrant the conclusion that the ripeness of an egg at the moment of its fertilization determines its sex. In explaining the results of a certain set of experiments in which the eggs of a female from one locality were fertilized with sperm from a male taken from a different locality, Hertwig further modifies his theory by the suggestion that in some cases the spermatozoön exerts a definite influence on sex-determination, although, as a rule, it is probable that "die Eier zur Zeit der Befruchtung sexuell in so hohem Grad determiniert sind, das der relativ geringe Einfluss des Samens gar nicht zur Geltung kommen würde."

Morgan ('08) has recently given a brief review of this work of Hertwig's on sex-determination and also a criticism of the results. He states that "Hertwig's attempt to connect his view with the ratio of nucleus to cell-plasm of the egg at different periods of its maturation can hardly be looked upon favorably, since in the frog's egg the nucleus as such has already disappeared when the egg leaves the ovary. The chromosomes are thereafter arranged on the equatorial plate of the first polar spindle. It is, however,

during this period that the degree of ripening is supposed to determine the sex of the egg." Morgan suggests that the results of Hertwig's later experiments seem to show that the male is responsible for sex-determination. The preponderance of one sex over the other would be explicable on the assumption that "more sperm of one kind, if two kinds exist, are injured or that internal processes may lead to the production of more functional sperm of one sex." It is unfortunate for this suggestion of Morgan's that the numerous investigations which have been made of the spermatogenesis of various species of amphibians have so far failed to show the slightest evidence of a dimorphism in the spermatozoa.

The frog offers much more favorable material than the toad for an investigation of the influence of the ripeness of the egg at the time of fertilization on sex-determination, since it is possible to extend the laying period of a frog over several days while the toad lays all of her eggs within a few hours whether she is separated from the male or not. Two different experiments were made with the eggs of *Bufo* in order to study this problem. In the first experiment a pair of toads were used that had been brought into the laboratory early in the morning of March 30, 1907. The female commenced laying at 10.45 A. M. of the same day. At 11.15 A. M. the toads were disturbed and the eggs already laid (Lot *A*) were removed to a jar of fresh water. The female began laying again at 12.05 P. M and a second set of eggs (Lot *B*) were removed at 12.25 P. M. The third laying (Lot *C*) began about 1 P. M. and was interrupted at 1.15 P. M.; while the fourth and last lot of eggs (Lot *D*) were deposited between 2.15 P. M. and 3 P. M. The laying period for this female, therefore, extended over about four hours. Four hundred eggs were taken from each lot for the purposes of the experiment, each set of eggs being put in a compartment of a large aquarium. The compartments were all approximately of the same size and each contained about the same amount of water and of plant food. All of the 1,600 individuals used in this experiment were, therefore, subjected to similar external conditions during the period of their development. There was no appreciable difference in the rate of growth of the tadpoles in the dif-

ferent compartments and the mortality was nearly the same in each lot. The results of this experiment are summarized in the following table.

TABLE II.

	Total Number Individuals.	Total Sex Ascertained.	Males.	Females.	Per Cent. of Females.
Lot *A*.....	400	149	70	79	53.02
Lot *B*.....	400	202	88	114	56.43
Lot *C*.....	400	133	56	77	57.89
Lot *D*.....	400	167	78	89	53.29
Total.....	1,600	651	292	359	55.14

The results of this experiment show a striking uniformity since in all four lots about the same proportion of the individuals in which sex was ascertained were females. The excess of females was slightly greater in the two middle lots (*B* and *C*) than in Lots *A* and *D*. This result would seem to support Hertwig's contention that eggs in the middle phase of ripening at the time of fertilization tend to produce a greater proportion of females. The extreme lots of the series (*A* and *D*) did not, however, give a correspondingly greater proportion of males as the theory demands. It is very probable, therefore, that the slightly greater proportion of females in the two middle lots has no especial significance. The comparatively short interval between the laying of the eggs in Lot *A* and of those in Lot *D* makes no direct comparison possible between the results of this experiment and those of Hertwig on *Rana*, since in some of Hertwig's experiments there was an interval of two or three days between the laying of the different lots of eggs by the same female. Normally the eggs of the frog, as well as those of the toad, are all laid within a short period of time. It would seem, therefore, as if in the above experiment a relatively greater number of males should have developed from the eggs of Lots *A* and *D* if, as Hertwig believes, the ripeness of an egg at the time of fertilization has any influence on its sex and if eggs fertilized in early or in late phases of ripening have a tendency towards male production.

All of the eggs used in this experiment should have been peculiarly favorable for the production of males according to Hertwig's theory, since, owing to the very early spring in 1907,

the eggs were laid fully one week earlier than is the usual breeding time for toads in eastern Pennsylvania. Of the 651 individuals in which sex was ascertained 359, or 55.14 per cent., were females. This result does not accord with Hertwig's view that the younger the egg at the time of its fertilization the greater its tendency towards male production. No eggs were obtained in the spring of 1907 from the end of March until the last week in April when several pairs of toads were brought into the laboratory and a large number of eggs laid and normally fertilized. According to Hertwig's theory these eggs were all overripe and should, therefore, have given a preponderance of males. To this series belong the eggs used in the experiment described in section 1. The results of the experiment in question, which are summarized in Table I., show that in the total of 600 individuals in which sex was ascertained there were 341 or 56.83 per cent. of females. This is about the proportion of females that is apparently normal for the species under natural conditions, and this normal sex ratio does not seem to be altered whether the eggs are laid the latter part of March or retained by the female until the end of April.

The second experiment that was made to test the influence of the ripeness of the egg at the time of fertilization on sex-determination was carried out as follows: A female which was isolated on the afternoon of April 5, 1908, began laying at 2 A. M. the following morning. She was at once killed by pithing and the body, unopened, placed in a moist chamber. At 9 A. M., seven hours later, the eggs were removed from the body of the female and artificially fertilized, sperm from two males being used. Although the eggs appeared perfectly good many of them did not fertilize, and the segmentation of the fertilized eggs was slow and in many cases abnormal. It is evident, from this fact, that post-mortem changes had already begun in the eggs at the time that they were fertilized and that it would not be possible to obtain normal embryos from eggs of *Bufo* that remained unfertilized much longer than seven hours after the death of the female. Although the eggs in this series were placed under the most favorable conditions possible many of them failed to gastrulate and the tadpoles that lived developed very slowly, large numbers of

them dying in the early stages of their development. Of the thousand or more eggs with which the experiment started only 372 tadpoles lived until it was possible to ascertain their sex, and in fully one half of this number the gonads had to be sectioned in order to make sure of the sex. Of the 372 individuals in which sex was ascertained 178 were males and 194, or 52.15 per cent., were females. The sex ratio found in these individuals which developed from "overripe" eggs agrees essentially with that found among the individuals in the first experiment where presumably the eggs were in very early phases of ripening at the time that they were fertilized. In both of these experiments an excess of females was produced, although, to accord with Hertwig's theory, there should have been an excess of males in each case.

The results of these experiments seem to indicate that in *Bufo* sex is not determined by the ripeness of the egg at the time that it is fertilized. Eggs fertilized normally within a period of four hours or artificially seven hours after the death of the female, eggs laid the last of March as well as those laid near the end of April, all produce a slight excess of females and thus give a sex ratio that is practically the same as that found among young toads that have recently completed their metamorphosis under natural conditions.

In connection with these experiments reference may be made to a theory of sex-determination recently elaborated by a physician, Dr. T. E. Reed ('07). Starting with the supposition that the ovum is hermaphroditic and that sex, which is a property or function of the ovum, is determined at the time of the fertilization of the egg, Reed then assumes that there is a sex cycle in the germ-plasm and that "this rhythm extends over a period of twelve hours, six being active or masculine, and six passive or feminine, and changes from day to day as do the tides. . . . The sex of the embryo is determined simply by the period through which the germ plasm happens to be passing when fertilization takes place." According to this theory if an egg is fertilized during the positive period, *i. e.*, when the tide is rising, the resultant embryo is always a male; if fertilization takes place during the negative period, when the tide is ebbing, the embryo is neces-

sarily a female. Reed gives no statistics regarding the working out of this hypothesis, but he states that it holds good for a score or more of cases in man and a number of cases in the breeding of horses and cattle. He does not explain, however, how the theory works in the case of such animals as dogs, pigs, rats, etc., which produce a number of offspring of both sexes at one birth. It can hardly be supposed that the fertilization of the ova in all of these animals invariably takes place "at the turn of the tide" when, according to this theory it might be possible for some of the ova to be fertilized during the positive (male) period and others during the negative (female) period.

An opportunity was offered to test this theory for *Bufo* with the lot of eggs used for the first experiment described in this section. On March 30, 1907, the tide was high at Philadelphia, where the experiments were being carried on, at 2.22 P. M. According to Reed's theory the six hours previous to this time woud form the positive period during which all eggs fertilized should develop into males. Of the eggs used for the experiment in question Lots *A*, *B* and *C* were laid and fertilized within the positive period and should, therefore, all have become males. Lot *D*, which was laid about the time of the turning of the tide, might be supposed to produce some females. This last lot, as a matter of fact, did not produce as great a proportion of females as did Lot *B*, which was laid at the middle of the positive period and therefore, according to Reed, at the most favorable time for the production of males. Results similar to these were obtained with another lot of eggs laid within the positive period. Reed's hypothesis, therefore, is not adequate to explain the determination of sex in *Bufo* however valuable it may prove to be as a theory of sex-determination in some of the higher mammals.

IV. The Influence of Temperature on the Determination of Sex in Bufo.

It has been held by several investigators that temperature has a decided influence on sex-determination ; a high temperature favoring the development of females, a low temperature tending towards male production. Most of the experiments which have seemed to give support to this hypothesis have been carried out with the

lower forms, such as *Daphnia* and *Hydatina*. The only experiments which have seemed to show that temperature has an influence on sex-determination in the vertebrates are those recently made by Hertwig on *Rana*.

In the first experiment which he made to study the influence of temperature on sex-determination Hertwig divided a bunch of eggs of *Rana temporaria* into two lots; one lot of eggs was allowed to develop at a temperature of 25° C., the other lot developed at a temperature of 13° C. The mortality among the tadpoles that were subjected to the higher temperature was exceedingly great. Out of a total of 200 individuals only 67 underwent metamorphosis, and of this number 4 were males and 63 females. The individuals placed in the cold water lived much better that the others, although the development of their genital organs was so greatly retarded that their sex could not be ascertained even at the time of metamorphosis. In a second series of experiments on the eggs of *Rana esculenta* Hertwig obtained the result shown in the following table.

TABLE III.

Water Temperature.	Males.	Females.	Per Cent. of Females.
22–30° C.	245	127	34.13
13–21° C.	282	54	16.07

Hertwig considers that the results of these two sets of experiments indicate that a high temperature favors the development of females, although he states that a number of individuals in which sex was ascertained is too small to give a decisive answer to the question. In the second experiment the females formed but 34.13 per cent. of the individuals that had developed in the warm water and but 16.07 per cent. of those that had been reared in the cold water. As this experiment stands it hardly seems to me to offer any evidence in support of the view that a high temperature favors the development of females. Both lots of eggs gave a large proportion of males and normally, according to the investigations of von Griesheim ('81) and of Pflüger, there appears to be an excess of females among both young and adult frogs. It would be interesting to know under what conditions the eggs used in

this second experiment were laid as this knowledge might help to explain the reason for the extraordinary excess of males.

Hertwig repeated this experiment in 1906 using eggs of *Rana temporaria*. None of the tadpoles developing at a temperature of 20° C. lived to undergo metamorphosis and therefore their sex could not be ascertained. Out of 340 larvæ developing at a temperature of 10° C., 74 lived to undergo metamorphosis. Of this number 38 were males, 23 females, and in 11 the sex was not ascertained although the individuals were probably males. Hertwig's conclusion from this experiment is that cold favors the development of males as heat favors the development of females.

The following series of experiments was made to study the influence of temperature on the determination of sex in *Bufo*. Five hundred eggs laid and normally fertilized in the laboratory on the morning of March 31, 1907, were divided into two lots: one lot of 250 eggs was placed in a greenhouse, heated by steam, where the temperature of the water in which the tadpoles were developing varied from 23°–30° C.; the remaining 250 eggs were placed in a tank in an unheated basement room where the temperature of the water in which the tadpoles lived ranged from 14°–18° C. during the daytime and was probably much colder at night. Both lots of tadpoles were given similar food and all of the conditions, excepting the temperature, were made as nearly alike as possible. For convenience in description this set of 500 eggs will be called Lot *A*. An unusually high temperature has apparently as injurious an effect on the eggs of *Bufo* as it has on the eggs of *Rana* since a great many of the tadpoles that were kept in water with a temperature of 23°–30° C. died before it was possible to ascertain their sex. The tadpoles that lived developed very rapidly and many of them had completed their metamorphosis by the first of June. The tadpoles kept in the cold water were vigorous and healthy, and comparatively few of them died. Their development was very slow, however, as compared with that of the individuals living in the warm water. At the end of May the largest of the tadpoles in this lot had a body length of but 8 mm. and their hind legs were still very small. None of these tadpoles underwent metamorphosis until the third week in June.

From a second lot of eggs laid in the laboratory on March 31, 1907, another 500 eggs (Lot *B*) were taken and divided into two sets of 250 eggs each as in the previous experiment. These eggs were placed under conditions similar to those of Lot *A*. The tadpoles in this series behaved in all respects like those in the first series as regards their rate of development and time of metamorphosis. The following table summarizes the results obtained with these two lots of eggs.

TABLE IV.

Water Temperature.	Number Sex Ascertained.	Males.	Females.	Per Cent. of Females
Lot *A* } 22–30° C.....	63	19	44	69.84
Lot *B* }	39	30	9	23.07
Lot *A* } 14–18° C.....	169	68	101	61.58
Lot *B* }	117	73	44	37.60

As shown in the above table the sex ratio of the individuals used in these experiments differs considerably from the sex ratio which is presumably normal for the species. The individuals of Lot *A*, whether they had lived in warm water or in cold water, produced an unusually large proportion of females; in the one case 69.84 per cent., in the other 61.58 per cent. of the individuals in which sex was ascertained were females. The most striking deviation from the apparent normal sex ratio is shown in the individuals of Lot *B*. Here the number of females is only 23.07 per cent. in the case of the tadpoles that had been reared in the warm water and 37.60 per cent. among the individuals that were subjected to a temperature of 14°–18° C. during the course of their development. The results of these experiments fail to support Hertwig's contention that a high temperature acting during the course of the development of the tadpoles favors the production of females, while a low temperature tends to produce a relatively greater proportion of males. To be sure one set of eggs (Lot *A*) developing at high temperature gave 69.84 per cent. of females which is a considerably larger proportion of females than is found among young toads under natural conditions; but another set of eggs (Lot *B*) developing under exactly similar conditions produced but 23.07 per

cent. of females. Just as striking a difference is shown in the case of the individuals that were reared in cold water. Lot *A* produced 61.58 per cent. of females, while Lot *B*, developing under similar conditions, gave but 37.60 per cent. of females. The very different sex ratios obtained with lots of eggs subjected to the same temperature conditions seem to indicate that sex in *Bufo* is not determined by the temperature of the water in which the tadpoles develop.

If sex is already determined in the egg and not influenced by external factors there is probably a considerable variation in the sex ratios of lots of eggs laid by different females. It might, therefore, be possible to explain the above results on the assumption that, by chance, batches of eggs were taken for the experiment which gave the extremes of possible normal variations in the sex ratio. This explanation is hardly a satisfactory one, especially in light of the results obtained in the experiment about to be described. The batch of eggs from which the individuals used in Lot *A* were taken was laid in water which ranged from 16°–18° C. during the two hours or more in which the eggs were being deposited and fertilized. The eggs used in Lot *B*, on the other hand, were laid in water which had a temperature of from 11°–13° C. during the period of their deposition. The results of the experiment at once suggested the idea that the temperature of the water at the time that the eggs were fertilized might possibly have some influence on sex ; a high temperature favoring the development of females, a low temperature that of males. The following experiment was made to determine the value of this suggestion.

On April 5, 1908, a pair of toads were placed in water which remained at a temperature of about 26° C. during the time that the eggs were being deposited and fertilized. All of the eggs that were laid fertilized readily at this temperature and all segmented in a normal manner. For convenience in description this set of eggs will be called Lot *C*. On the same date another pair of toads were placed in water which had a temperature of about 9° C. during the period in which the eggs were being deposited. All of the eggs in this set, which will be called Lot *D*, were also fertilized and all developed normally. There was,

therefore, a difference of about 17° C. in the temperature of the water in which these two sets of eggs were laid. The eggs of each lot, several thousand in number, were put in a large cement tank supplied with running water and containing plenty of food. The individuals in Lot *C* developed somewhat more rapidly than those in Lot *D* and they began their metamorphosis sooner. The experiment had to be discontinued on July 7, although a number of individuals in each set had not yet reached a stage of development when it would be possible to ascertain their sex. The results of the experiment, tabulated according to the weeks when the individuals died or completed their metamorphosis, is given in the following summary.

TABLE V.

	Lot *C*.			Lot *D*.		
Date.	No. Sex Ascertained.	Males.	Females.	No. Sex Ascertained.	Males.	Females.
May 24–June 6.....	126	66	60	34	18	16
June 7–13 ...	328	160	168	132	98	34
June 15–20 ...	790	344	446	808	524	284
June 22–27 .	697	255	442	924	422	502
June 28–July 7.....	237	128	109	185	119	66
Total...	2,178	953	1,225	2,083	1,181	902

As shown in the above table the sex was ascertained of 2,178 of the individuals that developed from the eggs laid in warm water. Of this number 1,225, or 56.24 per cent., were females, thus giving a sex ratio for Lot *C* that is little, if any, higher than that which is apparently normal for the species. The results of this part of the experiment, therefore, do not seem to favor the suggestion that a high temperature acting on the eggs at the time of their fertilization tends to produce a relatively greater number of females. There is the possibility, however, that this negative result may be due to the fact that in making the experiment a higher temperature was used than that which is the optimum for the production of the greatest number of females and that many more females would have developed had the eggs been fertilized in water with a temperature but slightly higher than that used in the former experiment with Lot *A*.

The sex ratio found among the individuals in Lot *D*, agreeing with that of Lot *B*, shows a considerable deviation from the sex ratio that is presumably normal for the species. In a total of 2,083 individuals which developed from eggs laid in water with a temperature of 9° C. there were 902 or 43.30 per cent. of females. Only once in the course of my investigations on sex-determination in *Bufo* have I found the proportion of females in a lot of individuals anywhere near as low as that found in Lots *B* and *D* in the above experiments. In studying the influence of nutrition on sex-determination in *Bufo* I found that in a total of 349 individuals that had been fed on mixed animal and vegetable food only 45.84 per cent. were females. Other lots of eggs laid by the same females gave as high as 59 per cent. of females however; and on summarizing the results obtained in the entire series of experiments it was found that the females formed 53.58 per cent. of the total number of individuals in which sex was ascertained. In the last experiment described there were 2,127 or 49.91 per cent. of females among the 4,261 individuals whose sex was ascertained. This fact strongly suggests that the sex ratio in this lot of individuals was affected by the conditions under which the eggs were laid.

The results of the experiments described in this section indicate, although they by no means prove, that the temperature of the water in which the eggs are fertilized has some influence on the determination of sex in *Bufo*. A high temperature appears to favor the development of females; a low temperature, on the other hand, seems to lead to the development of relatively more males. It is possible, as before suggested, that the results obtained in these experiments may be due to the fact that the sex ratio in *Bufo* is a variable one, the proportion of females ranging from 35–70 per cent. in lots of eggs laid by different females. If such is the case it surely is a strange coincidence that lots of eggs should have been taken for these temperature experiments which would give the greatest extremes in the sex ratio.

One serious objection that can be made to these experiments is that the eggs which were fertilized in warm water were laid by one female while the eggs fertilized in the cold water were laid by another individual. This gives an opportunity for the results

to be influenced by possible normal variations in the sex ratio in different lots of eggs. The two sets of eggs were fertilized by sperm from different males also and there is the possibility, as Morgan has suggested, that the male is responsible for sex-determination in amphibians. The last experiment was carried out in the manner described in order to obtain as large a number as possible of eggs that were fertilized normally at different temperatures, since it is not safe to draw conclusions from the results of experiments in which comparatively few individuals are used. Another series of experiments will be made next spring which, it is hoped, will determine definitely whether temperature can influence sex in *Bufo*. An account of the experiments already carried out is published in the hope that other investigators working in the same field may consider the suggestion here made as of sufficient value to warrant testing on any suitable material with which they may be working.

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THE INFLUENCE OF SOME AMINO-ACIDS ON THE DEVELOPMENT OF ECHINODERMS.[1]

A. P. MATHEWS.

While a great deal has been done on the influence of various external factors on development, little is known concerning the influence of those various metabolic products produced by the organism itself. It is probable that such products influence both the rate and character of development and it has long seemed to me that by varying them we could most surely reach our goal of an understanding and control of the mechanism of development. Accordingly I added to sea-water containing developing eggs of the sea-urchin, *Arbacia punctulata*, various amounts of cystin, leucin and tyrosin to see what changes would be produced in development.

The most interesting result was obtained with cystin. Cystin is the sulfur-containing amino-acid of the albumin molecule and has the composition, $C_6S_2N_2H_{12}O_4$. The sample I used was obtained by Mörner's method from horn. It is almost insoluble in cold water. One hundred cubic centimeters of sea-water were shaken for a moment with about a centigram of crystalline cystin and the mixture poured into a finger bowl with the undissolved cystin. The eggs, fertilized something less than an hour before, the time varying in different experiments, were then added and the eggs lay during development among the crystals of cystin at the bottom of the dish. At the same time similar transfers from the same lot of eggs were made to sea-water shaken in the same way but without the cystin. This was to throw out the possibility that aeration or some other factor was responsible for the results obtained.

The sea-urchin eggs this summer showed again, in many instances, the remarkable peculiarity recorded by Whitcher[2] and myself, a large number of eggs while living for several days not forming plutei, or but a small per cent. of irregular plutei, and the proportion of these going abnormally was increased by the

[1] From the Marine Biological Laboratory, Woods Holl.

[2] Mathews and Whitcher, *Amer. Journal of Physiol.*, VIII., 1903, p. 300.

act of transfer. The effect of cystin on such eggs was indeed most striking. Whereas in the controls hardly a pluteus was to be found and these few were generally abnormal, the cystin eggs nearly all formed normal plutei. The effect, also, of the cystin on the development of the more normal eggs was perfectly invariable. The cystin eggs always showed a decided acceleration of development, so that they were plutei while the controls were still gastrulæ. The acceleration was visible from about the fourth division on. Plutei formed in the cystin solutions within 24 hours of fertilization. The cystin not only accelerates the process of pluteus formation, but a gradual acceleration of development takes place from the start.

We have, therefore, in cystin a substance which acts most beneficially on *Arbacia* development, particularly in those cases where the development is for some reason abnormal. It resembles in this particular the action of alkalies as recorded by Loeb[1] and pilocarpine in the case of star-fish eggs.[2] The conclusion is not of course justified that the abnormality is due to a deficiency of cystin possibly produced normally by intracellular digestion, although such a possibility is not in itself improbable. If the eggs are left in contact with cystin their death takes place after 48 hours, but if they are transferred to fresh sea-water after 24 hours in the cystin water, their subsequent development does not appear to be interfered with. They develop normally thereafter.

Toward the star-fish eggs cystin proved to be toxic and the only effect noticed was the early death of the egg. No acceleration was produced in the development of the mollusc, *Cumingia*, by the addition of cystin. The action appears, therefore, to be rather specific for *Arbacia*.

I also tried the action of tyrosin and an impure leucin from horn upon *Arbacia* eggs. The tyrosin, while not very soluble, is more soluble than cystin and accordingly more of it is in solution to act on the eggs. A saturated solution was at first used, the sea-water always containing undissolved tyrosin crystals. The effect of such a saturated solution on the development of *Arbacia*, when the experiments were tried in the same way as those with cystin, was invariably harmful; development was retarded and the eggs

[1] Loeb, *Archiv für Entwickelungsmechanik*, 1898, VII., p. 631.

[2] Mathews, *Amer. Journal of Physiol.*, VI., 1901, p. 207.

ultimately killed. With weaker solutions the results were uncertain. In some cases the development was slightly accelerated, but more often it was either not affected or else retarded.

Impure leucin prepared from horn proved very toxic, possibly owing to some impurity, but possibly to the leucin itself. The effect of a weak solution is extremely interesting in that it arrested development without killing the eggs. Particularly it prevented development beyond the blastula stage. The embryos were unable to escape from the membranes; they remained without farther development in these membranes but still alive, for 24 to 72 hours. The color gradually disappeared until they were as colorless as star-fish larvæ. I took some of them out to fresh sea-water after being thus blastulæ for 30 to 40 hours, to see whether their subsequent development would be interfered with. After a time the embryos emerged from the membranes and swam about; they lived for days and developed into all sorts of fantastic embryos. Some of them were totally unlike *Arbacia* larvæ. In many, evagination of the entoderm instead of invagination, took place. A few developed a ciliated band in the shape of the star-fish *bipinnaria* larvæ which they resembled far more nearly than they did *Arbacia*. These forms, however, never developed a well marked entoderm like that of the star-fish, nor did the mouth and anus invaginate. Another highly interesting form was perfectly spherical with a single ciliated band about the middle and swimming rapidly. It looked in its external form like a very small trochophore. Obviously the sample of leucin which was used checked some particular development but allowed other processes to go on so that the embryos lived but developed in a widely different direction from normal. I know of no means of proving so clearly the infinite number of different embryonic forms resident in each egg. Possibly an examination of these different forms might be of decided interest, and I hope that some embryologist may think the subject worth investigating since it lies apart from my own field. I was unable to make all the embryos abnormal in one direction by leucin, but I did not particularly investigate this possibility.

These experiments show that the products of intracellular protein digestion may be very important in determining development.

EXPERIMENTAL CONTROL OF CERTAIN REGULATORY PROCESSES IN HARENACTIS ATTENUATA.

C. M. CHILD.

In my study of form regulation in *Cerianthus*[1] it was shown that distension of the enteron with fluid was an important factor in regulation in this form. More recent work on *Harenactis attenuata* an actinian inhabiting the tide flats near San Diego, Cal.,[2] has confirmed my earlier conclusions and has afforded certain striking examples of the importance of distension for the persistence of parts. The results of my work on *Harenactis* will be presented elsewhere, but it seems desirable to give a brief account of certain experiments at this time since they illustrate so clearly not only the importance of distension but the possibility of experimental control of the course of regulation.

These experiments are concerned with the production of partial discs proximal to the original disc in consequence of cuts part way through the body. In my earlier experiments of this kind on *Cerianthus*[3] it was found that in all cases the region directly distal to the partial disc underwent atrophy and disappeared and the partial disc gradually migrated toward the distal end of the body and finally took the place of the atrophied portion. I pointed out that the atrophy in these cases was very evidently due to insufficient distension or absence of distension in the part concerned and showed that the method of closure of the wound in such cases resulted in isolation of the enteric cavity of the atrophying region both from the exterior and from other portions of the enteron. Although the entrance of water through the wall of the body is apparently possible to a greater or less ex-

[1] Child, "Form Regulation in *Cerianthus*," I.–IX., BIOL. BULL., Vols. V.–VIII., 1903–5.

[2] Child, "Form Regulation of *Harenactis attenuata* in Altered Environment," BIOL. BULL., Vol. XVI., No. 1, 1908.

[3] Child, "Form Regulation in *Cerianthus*, VIII., Supplementary Partial Discs and Heteromorphic Tentacles," BIOL. BULL., Vol. VIII., No. 2, 1905.

tent, the usual degree of distension cannot be maintained in this way and in the absence of the functional stimulus produced by distension atrophy occurs.

In the case of *Harenactis* the occurrence or non-occurrence of atrophy depends upon the conditions of the experiment and the result can be controlled.

The possibility of experimental control in *Harenactis* depends on the position of the mesenterial ostia in the œsophageal region. The ostia are readily seen in anæsthetized specimens opened under water. Their approximate position is indicated by the small dotted circles on each side of the œsophagus in Fig. 1. This figure represents an individual in the extended condition characteristic of the normal habitat, a vertical burrow in the sand. When the animals are kept without sand extension is never as great as in the burrow and the animal gradually becomes incapable of any considerable extension.[1] Under these conditions the ostium appears to lie much nearer the distal end than in Fig. 1. Each mesentery possesses an ostium, consequently the inter-mesenterial chambers are in direct communication with each other about the whole circumference of the œsophageal region.

FIG. 1.

I have been unable thus far to discover the ostia in *Cerianthus* or to find references to them in the literature, so far as it is accessible to me. But whether they are present or not my experiments show very clearly that distension of the region directly distal to a lateral incision in the œsophageal region does not occur. The most casual examination of specimens after healing of the wound produced by such an operation is sufficient to show that this region is either completely collapsed or only very slightly distended.

[1] Child, "Form Regulation of *Harenactis attenuata* in Altered Environment," BIOL. BULL., Vol. XVI., No. 1, 1908.

In *Harenactis*, as in *Cerianthus*, the healing of the wound made by a lateral incision in the œsophageal region and deep enough to involve the œsophagus occurs in the manner indicated in Fig. 2. The cut surfaces of the body-wall and the œsophagus unite with each other both distal and proximal to the cut so that a new lateral opening into the œsophagus is formed, its extent depending on the extent of the incision. The factors concerned in this method of closure will be discussed elsewhere: at present we are concerned merely with the fact of closure in this manner. After healing of the wound tentacles appear proximal to the opening, *i. e.*, on the distal end of the region below the opening, their number depending on the number of inter-mesenterial chambers which were cut across by the incision. So far *Harenactis* and *Cerianthus* are alike. In *Harenactis*, however, but not in *Cerianthus*, heteromorphic tentacles appear somewhat later at the proximal end of the region distal to the cut (Figs. 3 and 5). As will be shown elsewhere heteromorphic tentacles appear much more readily in *Harenactis* than in *Cerianthus* under various conditions. After these lateral incisions in the œsophageal region they always appear. If the old disc is removed at the time the lateral incision is made tentacles develop about the whole circumference of the distal end of the body. My figures represent cases in which the original disc was left intact: the final result is the same whether the old disc is removed or not.

FIG. 2.

After an operation of this kind in *Harenactis* there exists then a region distal to the lateral opening in which the inter-mesenterial chambers are completely shut off from other portions of the enteric cavity in the longitudinal direction. The presence or absence of communication in the transverse direction, however, depends on the position of the incision: if this was made distal to the ostia (*a*, Fig. 1) each chamber is completely isolated from the others and from other portions of the enteron; if, on the other hand,

the incision was proximal to the level of the ostia then all the chambers distal to the incision are in communication with each other and with the enteric cavity in general. In the first case distension of other portions of the body with water has no effect upon this region, but in the second, distension of other parts is accompanied by distension of this region.

In *Harenactis*, as in *Cerianthus*, a considerable degree of distension exists when the animals are undisturbed, consequently body-wall, tentacles and mesenteries are subjected to a certain degree of mechanical tension as a characteristic feature of normal life. The following experiments show very clearly the importance of this distension as a factor in determining the persistence or atrophy of parts.

Fig. 3 is a diagrammatic longitudinal section of an animal ten days after a lateral incision just distal to the level of the ostia (*a*, Fig. 1). Tentacles have developed proximal and distal to the opening as usual, but the whole region distal to the opening is

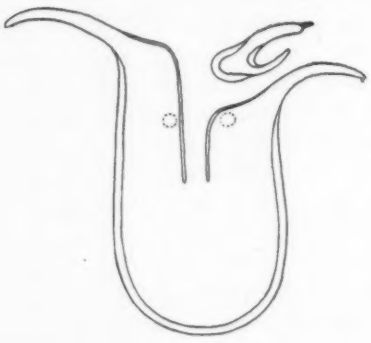

FIG. 3.

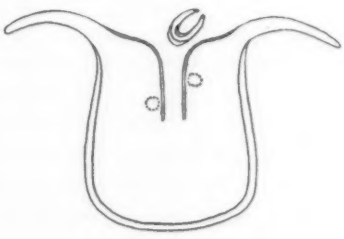

FIG. 4.

much less distended than other parts of the body. The old tentacles at the distal end of this region are undergoing atrophy, as is indicated by their length as compared with the tentacles of the other side of the body and by the degenerating region at the tip: the heteromorphic tentacles at the proximal end of this region are small and short; on the other hand, the tentacles proximal to the incision are nearly as long as the old tentacles on the other side of the body.

Observation of such cases in distended and collapsed condition establishes the fact that changes in the degree of distension of other regions do not alter the distension in the region directly distal to the incision. In all such cases this region acquires a certain degree of distension after healing of the wound, undoubtedly in consequence of the passage of water through the walls, but this distension gradually decreases as time goes on until finally collapse is complete.

The condition of such animals three to four weeks after the operation is indicated in Fig. 4. The region distal to the cut has undergone rapid atrophy and the partial disc below it has attained the same level as other parts of the old disc. The atrophied region is now completely collapsed and in an oral view of the animal forms merely a narrow strip across the disc with a mouth opening on each side of it.

A little later this strip breaks and its two ends become still further reduced and soon undergo complete resorption or in some cases drop off, leaving a normal disc.

The result is very different when the incision is made proximal to the level of the ostia, *e.g.*, at *b*, Fig. 1, or any level proximal to this within the œsophageal region. In such cases (Fig. 5) the old tentacles distal to the incision do not undergo atrophy and the heteromorphic tentacles become almost or quite as long as the others. Moreover, examination of the specimen shows at once that the whole region distal to the incision is as fully distended as other parts of the body when the animal is undisturbed, and when contraction and expulsion of water occurs this region takes part in the change, becoming distended again when other parts of the body distend. Fig. 5 shows an individual of this kind two weeks after the operation, a time when atrophy has already begun in cases of operation distal to the ostia. In my experiments a considerable number of these cases were kept for four and a half months. During this time decrease in size of the whole body occurred since the animals were not fed and in most cases both the oral and aboral tentacles of the region distal to the incision became somewhat shorter than the others, though there was no visible atrophy such as occurs in the other cases (Fig. 3). Fig. 6 shows an individual of this kind one hundred

and thirty-six days after operation. The region distal to the incision is somewhat shorter than in the early stages but it still shares in the distension of the body, the tentacles are fully functional and the tissues appear to be in perfectly healthy condition. The termination of my stay at La Jolla made further observations impossible, but there can be little doubt that this region would have remained intact and normal almost or quite as long as other

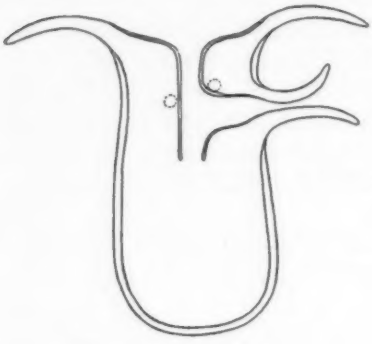

FIG. 5.

FIG. 6.

portions of the body. The fact that the tentacles of the region in question are often somewhat shorter than others is probably to be accounted for by nutritive conditions. The animals are undergoing slow starvation and apparently the material of the more proximal regions is used in greater or less degree in maintaining the more distal regions. But the region distal to the incision is only indirectly in organic connection with the proximal regions and therefore, in all probability, uses up its own material somewhat more rapidly than other portions of the body.

These two series of experiments seem to me to demonstrate that the conditions resulting from distension of the enteric cavity with fluid are necessary for the persistence of the body-wall and tentacles. When the incision is made distal to the level of the ostia the region distal to it cannot share in the distension of other parts and consequently undergoes complete atrophy in about a month. When the incision is made proximal to the level of the ostia this region shares in the distension of other parts and shows

no appreciable degree of atrophy within four and one half months. Discussion is unnecessary.

In a number of cases the lateral incision was made somewhat obliquely so that one end of it was distal to the level of the ostia, the other proximal. In these cases the part in which ostia were present became distended after healing, produced tentacles almost or quite as long as the others and persisted during four and one half months: the part from which ostia were absent became slightly distended after healing, produced short tentacles, then underwent gradual collapse and complete atrophy within a few weeks. Consequently these animals in their final condition possessed a structure arising from one end of the lateral incision and ending free, since the part which originally connected it with the other end of the incision had atrophied, and bearing tentacles on its oral and aboral surfaces. In certain respects these cases are the most interesting of all, since the two different results can be observed in a single animal.

To sum up: it is possible to determine experimentally whether persistence or complete atrophy of certain parts of the body of *Harenactis* shall occur: the factor determining the result is the distension of the enteric cavity with fluid or the absence of such distension.

HULL ZOOLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
December, 1908.

POLARITY AND BILATERALITY OF THE ANNELID EGG. EXPERIMENTS WITH CENTRIFUGAL FORCE.[1]

FRANK R. LILLIE.

In a previous paper (Lillie, '06) I attempted to show that the direction of polarity of the egg of *Chætopterus* is not modified by any experimental redistribution of the visible elements, nucleus and granules, and I therefore concluded that polarity is a property of the ground substance of the protoplasm. In the present paper I propose to examine the grounds for this statement, to attempt to show that bilaterality comes in the same category, and to examine the conceptions of polarity and organization that naturally result.

I. Polarity.

Polarity manifests itself first in the egg of *Chætopterus* when the ovogonium becomes an ovocyte and takes its place in the ovarian epithelium. It has then a free pole and an attached pole, which have different physiological relations and exhibit different morphogenic activities. When the egg becomes free from the epithelium it is found that the original free pole becomes the animal pole in development, and the attached pole becomes the vegetative pole. The developmental processes take place with reference to these poles; thus the various granules of the egg rearrange themselves in a definite polarized fashion, the polar bodies have an absolutely determined relation to polarity, cleavage takes place and the various organs arise in definite topographical relations to the polarity.

In the paper already referred to I showed that if the egg is centrifuged with sufficient force the result is to separate the protoplasm into three strata, viz.: a central grayish cap, an intermediate hyaline or clear band which contains the nucleus

[1] The substance of a part of this paper was presented before the joint session of the American Society of Zoölogists, and Section F — Zoölogy — of the American Association for the Advancement of Science, held in Chicago, December, 1907. (An abstract was printed in *Science*, Vol. XXVIII., No. 702, pp. 905–907, June 12, 1908.)

or spindle, and a distal yellow hemisphere which contains the larger granules known as yolk (Fig. 1). The results prove that the eggs are not oriented in the centrifuge with reference to the original polarity, and therefore the axis of stratification bears all

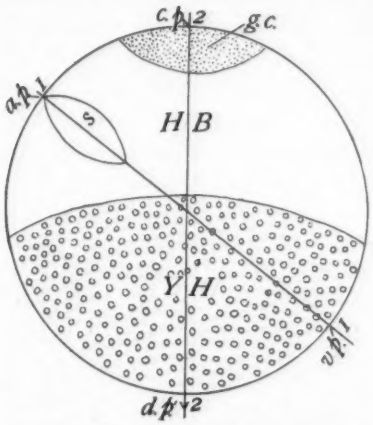

FIG. 1. Diagram of a centrifuged egg of *Chætopterus*. *a.p.*, animal pole; *c.p.*, central pole, or pole turned towards the axis of the centrifuge; *d.p.*, distal pole, or pole turned away from the axis of the centrifuge; *g.c.*, gray cap; *H.B.*, hyaline or clear band; *s.*, maturation spindle; *v.p.*, vegetative pole; *Y.H.*, yellow, yolk-bearing, or distal hemisphere; *1–1*, primary axis; *2–2*, secondary axis.

possible relations to the polar axis of the egg. We may call the original polar axis the primary axis, and the line through the center of the egg in the direction of the centrifugal force the secondary axis (Fig. 1).

If such eggs are fertilized the polar globules of different eggs form at any point of the surface with reference to the secondary axis. Cleavage takes place with reference to the axis defined by the polar globules, as in the normal egg, and is approximately normal in form. It therefore follows that the distribution of yolk and other substances induced by the centrifuge is substantially immaterial so far as the form of cleavage is concerned. The effect of abnormal distribution of yolk and other substances on the differentiation of organs is not considered here.

On the basis of these results I concluded that the polarity of the ovum had not been affected by centrifuging, and that the

polar bodies appear in the same position that they would have normally, that is, in the primary axis; in other words, that no redistribution of the granules or nucleus affects the direction of polarity of the egg. If this be true, then the final conclusion stated in that paper, that polarity is a function or property of the ground substance of the protoplasm, certainly follows.

This conclusion is of fundamental importance for the entire theory of development, and its basis therefore requires the most careful examination. One thing is clear at the start, viz.: that this point of view affords a complete explanation of the phenomena, because the egg is polarized prior to centrifuging, and the centrifugal force acts in all possible directions with reference to the polar axis;[1] and this agrees with the fact that the polarity that appears after centrifuging also lies in all possible directions with reference to the axis of stratification. The question is simply this: Is the polarity as marked by the polar bodies and cleavage planes the same polarity in the normal egg and after centrifuging? Does any other hypothesis agree so well with the observed facts, and if so, is it for any sufficient reason to be preferred to this hypothesis?

An alternative hypothesis can lie only in one direction, viz.: that polarity is determined by any point on the surface of the egg to which the maturation spindle happens to go after cen-

[1] The proof of this statement is as follows: In the stage used for the experiments (except where otherwise stated) the first maturation spindle is fixed at the periphery at the stage of the mesophase. Its outer pole marks the center of the animal pole; this point is also characterized by an entire absence of the ectoplasm, which covers the remainder of the surface of the egg as described in a previous paper ('06). If sections of centrifuged eggs are examined, the maturation spindle is found in about half of the eggs still attached to the surface, at any point in the hemisphere containing the hyaline band. At first sight this would indicate that the eggs oriented themselves in the centrifuge to a slight extent, though it is clear that the animal pole is in some cases 90° removed from the ends of the secondary axis. But the fact that fixed spindles are not found in the distal (yolk-laden) hemisphere after centrifuging has another explanation: about half of the eggs after centrifuging have free spindles (*i. e.*, not attached to the surface), which always lie in the hyaline band, and these represent those eggs whose original animal pole lay in the distal hemisphere in the centrifuge. (Cf. Fig. 8, *C* and *D*.) The inrush of yolk into the distal hemisphere has torn the spindles loose from the periphery, and their specific gravity has carried them into the hyaline band; this interpretation of the free spindles is proved by the fact that many of them migrate into the distal hemisphere later in the process of forming the polar bodies (Fig. 2).

trifuging. Now, assuming that there is no polarity of the ground substance, as this hypothesis requires, the spindle would naturally follow the shortest path to the surface, for this would usually be the path of least resistance. In other words, the position given the nucleus or the spindle by the centrifugal force would determine the polarity in all cases, because its position is always excentric. However, this is not the case, as the following facts demonstrate :

1. Most of the experiments on the egg of *Chætopterus* were made before fertilization at the stage of the mesophase of the first maturation spindle. All the ripe eggs of this animal attain this stage without fertilization, and the spindle comes to rest and remains at the mesophase unless the egg be fertilized or otherwise effectively stimulated (Fig. 4). The spindle is attached to the surface and its axis is in line with the axis of the egg. If the direction of the centrifugal force is such that the spindle lies in the central hemisphere, *i. e.*, in the half of the egg towards the post, it may remain attached, but is often detached from the surface, and in such cases it always lies in the clear band. If the spindle pole of the egg lies in the distal hemisphere during centrifuging, it is always detached from the surface and comes to rest in the clear band. The result therefore is always the same so far as the position of the spindle is concerned : the spindle lies invariably in the clear band after centrifuging, either attached to the surface, or free.

Nevertheless, the polar bodies may form at any point in the yolk hemisphere, as is demonstrated by study of the living eggs, and also by sections of series of eggs preserved during the maturation period after centrifuging. Figures of such living eggs are given in the paper already referred to, and a section of such an egg is reproduced here (Fig. 2). When one considers the resistance that has to be encountered by the spindle in making its way through the densely packed yolk-granules of the distal hemisphere, and remembers that a shorter path to the surface through hyaline protoplasm was open, it is obvious not only that the spindle does not follow the shortest path to the surface, but also that a factor of great intensity has been in operation.

I stated in my previous paper ('06) that the formation of polar

globules in the yolk hemisphere is rarer than their formation in the hyaline hemisphere, and was inclined to regard this as evidence that there was a tendency for the eggs to orient themselves in the centrifuge with the vegetative pole away from the center. But I no longer believe that this is the case. The relative rarity of maturation at the distal pole of centrifuged eggs must be due to a different cause, and this is the great resistance of the yolk

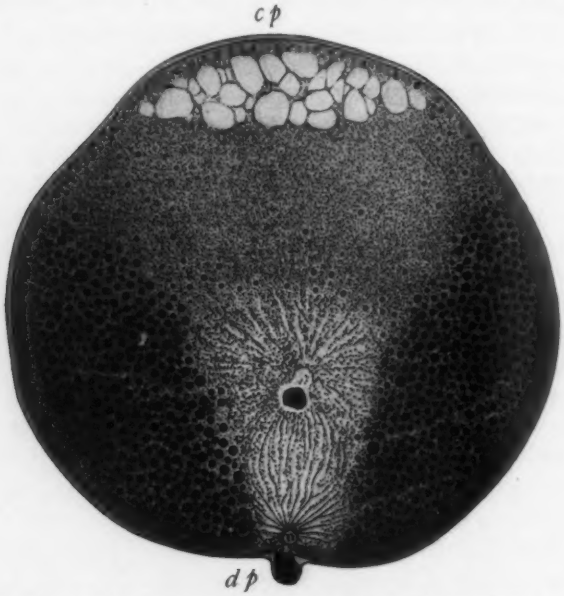

FIG. 2. Egg of *Chætopterus* centrifuged 2,300 revolutions in one minute at a radius of 13 cm., 3:57 P. M., June 27, 1906; fertilized 3:58 P. M., killed 4:27 P. M. The formation of the polar globules has taken place at the distal pole. The maturation spindle migrated through the entire yolk-mass in the distal hemisphere to reach this position. The sperm-nucleus surrounded by radiations is seen a short distance above the egg-nucleus. The vacuolated area above is the gray cap. *c.p.*, central pole (secondary axis); *d.p.*, distal pole (secondary axis).

mass to penetration by the spindle. This conclusion is clearly demonstrated by study of series of sections of eggs preserved at regular intervals after centrifuging and fertilizing, which show that many such eggs fail to form the polar bodies at all, or are much delayed, or undergo their maturation divisions within the egg.

2. An even more conclusive argument is afforded by centrifuging ovocytes with intact germinal vesicle. As shown in the paper already referred to, the large germinal vesicle passes to the central pole; it is the lightest constituent of the egg. If, therefore, the position of the nucleus determines polarity, the central pole should become the animal pole in such cases; the polar bodies should form here. I made a number of experiments on this subject in 1906 and 1908. The difficulties found in interpreting these experiments are: (*a*) that the stratification induced by low centrifugal powers is relatively slight in the stage with intact germinal vesicle, apparently owing to greater vicosity of the ground substance at this stage, and (*b*) that it does not persist to the time of formation of the polar bodies, owing to the process of polarization of granules that follows the rupture of the germinal vesicle, which I described in a previous paper. However, by following individual eggs in which the position of the centrifuged germinal vesicle is known, the relation of the polar bodies to the position of the germinal vesicle can be readily ascertained. It is then found that there is no fixity of relation whatever; in other words, the maturation spindle, which forms at the site of the germinal vesicle, may migrate through the entire diameter of the egg to become fixed at the opposite pole, or perform any lesser migration. The same thing is demonstrated if the eggs are submitted to very high centrifugal powers; under these circumstances the stratification persists up to maturation in some eggs to a sufficient degree to demonstrate positively that the polar bodies form in all positions with reference to the centrifuged position of the germinal vesicle.

Thus one cannot determine the place of formation of the polar bodies by simply throwing the nucleus to the surface of the egg.

A third argument may be based on comparative grounds. Very many cases are known in which polarity determines the position of the nucleus; the literature on fertilization is full of such cases. I know of no observations that tend to show determination of polarity by position of the nucleus.

The results, then, certainly demonstrate that polarity is not a result of the position of the nucleus or of any configuration of granules. It follows that it is a property of the ground sub-

stance; and when one considers carefully the data presented, one cannot avoid the conclusion that the direction of polarity is unmodified by the centrifugal powers employed. It must therefore depend on some configuration or heterogeneous physical or chemical properties of the ground substance established early in the history of the egg, and which is not essentially disturbed by centrifuging. This subject will be examined in the third part of the present paper.

The conclusion stated above was clearly presented in my paper of 1906. Since then Morgan (1908) has come to the same conclusion for the egg of *Arbacia*. In his last paper Morgan furnishes a satisfactory demonstration that polarity is unmodified in the egg of *Arbacia* by centrifuging, by showing that the micropyle which bears a constant relation to polarity in the normal egg has the same constant relations to the polarity of centrifuged eggs, as proved by formation of the micromeres and archenteron at the pole opposite the micropyle whatever the direction of the centrifugal force, and therefore whatever the position of the centrifugal zones and the form of the cleavage modified (in *Arbacia*) by these zones. I therefore take Morgan's result to be a complete confirmation of my original and present position.

The demonstration that the direction of polarity is unaltered by centrifuging involves the assumption that the configuration of the ground substance remains the same. In other words, that there is a definite architecture in the ground substanee, which is the basis of the localization pattern in normal development (see *Science*, N. S., XXVII., June 12, 1908). This also involves a new conception of protoplasmic streaming as seen in ova, for this must be interpreted as granule movement exclusively, and not actual flowing. Similarly, the movements produced by the centrifuge cannot be mass movements of entire protoplasmic areas, but only granule movements through the ground substance. In the third part of the present paper, I shall consider these conclusions with some care.

Observations on Nereis.

In the summer of 1908, I had the opportunity of comparing the eggs of *Nereis limbata* with reference to these points, and I

found that, just as in *Chætopterus*, the place of formation of the polar globules is independent of centrifugal stratification, and that the cleavage follows the polarity marked by the polar globules and is not modified in its direction by the position of the yolk arbitrarily imposed by the centrifuge. Morgan has shown the same thing for the lamellibranch, *Cumingia* (*Science*, Vol. XXVII., p. 496, 1908), so that now we have at least four forms in which this principle is firmly established.

II. Bilaterality.

The second main question that I propose is whether or not bilaterality comes under the same category as polarity. And this resolves itself into two questions: (1) Is bilaterality, like polarity, a function of the ground substance, and (2) When does it arise?

1. The first morphological evidence of bilaterality in the eggs of *Chætopterus* and *Nereis* is found in the first cleavage. The spindle takes an excentric position, and the cleavage is therefore unequal; the smaller cell is anterior and the larger cell posterior in position. The course of the entire development is thus determined not only with reference to the bilateral axis, but also with reference to the proportions of embryonic organs.

If the bilaterality were a result of a kind of equilibrium of the protoplasmic inclusions (nucleus and granules), it is clear that the variations in position of the nucleus and granules produced by centrifugal force should involve change of direction of bilaterality. If, on the other hand, bilaterality is a function of the ground substance, changes in the position of the nucleus and granules should not materially affect the direction of bilaterality, though it might conceivably modify the inequality of the cleavage which is our index of bilateral organization. However, if the normal proportions of the first two cells and the meridional direction of the cleavage plane are maintained in centrifuged eggs in spite of the abnormal distribution of granules between cells that would be thus induced, no other evidence would be necessary to show that the seat of bilateral organization is in the ground substance.

This is in fact the case, both in *Chætopterus* and *Nereis*: Whatever the distribution of yolk and granules induced by centrifuging,

with reference to the primary axis, the first cleavage is invariably truly meridional, and the proportions of the cells are usually normal or approximately so. In some cases the small cell is packed full of yolk granules, in other cases practically devoid of them; nevertheless, the first cleavage is substantially unaffected as to direction or proportions by the direction of stratification (see Lillie, 1906, p. 199).

2. Is the bilateral organization of the egg a property of the primary ovocyte like polarity, or does it arise in the course of development after rupture of the germinal vesicle? The evidence seems to me in favor of the latter proposition for the following reasons:

(*a*) If the ground substance is bilaterally organized before maturation there should be morphological evidence of it, as there is of the existing polarity. But there is none whatever; the structure of the egg is radially symmetrical in the plane at right angles to the polar axis until the close of maturation. After the union of the germ nuclei, the bilateral symmetry comes to expression slowly by granule movements that cause a preponderant distribution of the yolk granules on one side of the egg, which becomes the posterior side, and by corresponding displacement of the yolk-free protoplasm and spindle towards the opposite side of the egg, which becomes the anterior side. This is the case in both *Chætopterus* and *Nereis*, as well as in *Unio*. If the conditions causing such displacement existed prior to maturation, one would expect that they would become effective at an earlier period.

(*b*) In the summer of 1908 I found that it was possible to break up the eggs of *Chætopterus* into fragments of varying sizes by high centrifugal force. The protoplasm of this egg is much more fluid than that of any other egg I have tested; in consequence, the egg elongates even with relatively low centrifugal speed; and with very high speed the eggs break apart along the lines of stratification. Thus one obtains numerous perfectly hyaline pieces composed of the substance of the clear band, and others densely packed with yolk granules. The nucleus is almost invariably contained in the former kind, but it may occur in the latter, owing to the fact that at the time of centrifuging the matu-

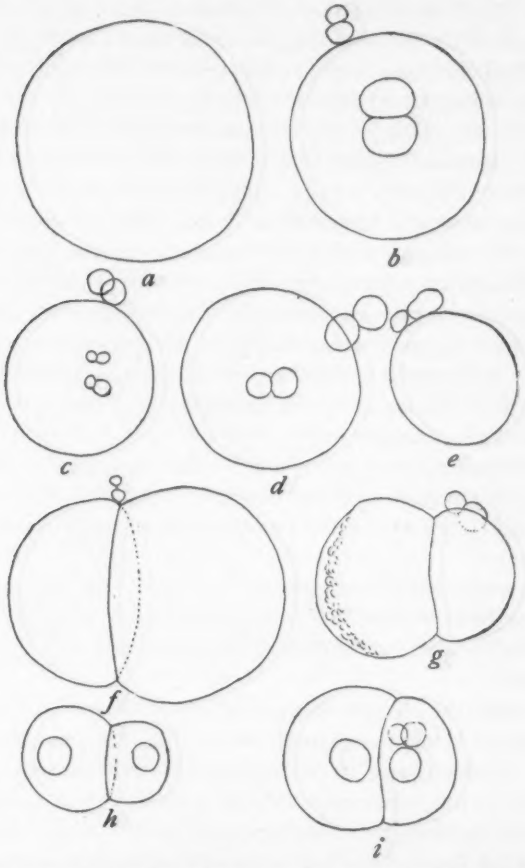

FIG. 3. Maturation and first division of hyaline fragments of the eggs of *Chætopterus*, obtained by centrifuging before fertilization. *a*, outline of normal egg for size-control; *f*, outline of normal two-celled stage for size-control; *b*, *c*, *d*, *e*, *g*, and *h*, hyaline fragments of various sizes from a single experiment; *i*, a similar fragment from another experiment. Fragment *g* contains a small quantity of yolk.

The eggs break apart along the planes of stratification with a high centrifugal force, and further subdivision may occur so that the fragments are variable in size.

It will be noted that the polar globules are abnormally large in the fragments (cf. Fig. *f*, control). The proportions of the first two cells are, however, approximately normal, Figs. *g*, *h*, *i* (cf. *f*, control).

All figures drawn with the camera at a magnification of 780 diameters, and reduced one half.

ration spindle is attached to the surface, and may thus be separated off in the yolk-bearing end of the centrifuged egg, if it lies in a distal direction in the centrifuge and is not torn loose from its attachment before rupture of the egg takes place. But while the majority of large hyaline pieces contain the maturation spindle and form polar bodies after fertilization, this happens very rarely indeed in yolk-bearing pieces.

I was interested to determine if the cleavage of the hyaline pieces was unequal, and, if so, whether or not the proportions of the cells approximated the normal. This is indeed the case; in most pieces taken before fertilization, that segment, the segmentation is unequal and in substantially the normal proportions. Seeing that the inequality of cleavage of the normal egg is an unfailing index of bilaterality, the same phenomenon in the parts must be interpreted in the same way.

The unequal cleavage of such hyaline parts absolutely devoid of yolk is a most striking phenomenon. There is great variation in the absolute size of such parts, but the cleavage remains proportional.

The parts vary not only in size, but also in the regions of the egg that they represent. The latter conclusion obviously follows from the fact that the direction of the centrifugal force is a matter of chance.

To show the strong tendency of pieces of the hyaline band to segment in normal proportions the following result may be cited. In experiment 10 *F*, August 18, 1908, a quantity of eggs taken from the female at 10 A. M. was allowed to stand in sea water without fertilization until 10:40 A. M. They were then centrifuged about 7,500 revolutions in one minute with a radius of 6 cm. and were thus broken up. The material was fertilized at 10:43 and allowed to develop to the two-celled stage, then fixed, stained and mounted entire in balsam. The impression of the preparation is that in practically every segmented hyaline piece the proportions of the cells are approximately normal. To remove the personal equation as far as possible, I marked a ring on the cover slip, and drew every segmented hyaline piece in it. Of 26 pieces so drawn, none had divided equally; in four pieces of the 26 the small cell was proportionally larger than normal,

and the remainder were substantially normal in the relative sizes of the two cells, although the smallest piece was about one eighth the volume of the entire egg, and the others ranged in size up to about one third the bulk of the normal egg. The pieces in which the small cell was too large relatively were the largest pieces and contained some yolk.

Now if bilateral symmetry existed prior to fertilization, one would expect parts of the bilateral egg to vary greatly in the proportions of the first two cells, because a fractional bilaterality and not a whole bilaterality would exist in each part. I therefore conclude that bilaterality has developed subsequent to fertilization. And this conclusion must be true *for the parts* even if it were only a question of regulation of a preëxisting bilaterality (of which there is no evidence). But if bilaterality can develop in the parts there is no reason for assuming its prior existence in the whole ; and its origin must be regarded as a truly epigenetic process. Brachet makes a similar argument for the egg of the frog (*Roux's Archiv*, XXII., 3, 1906).

III. The Organization in the Ground Substance. Examination of the Conceptions of Polarity and Bilaterality.

The hypothesis that the nature and direction of polarity and bilaterality are unaltered by centrifuging involves the assumption that the arrangement of the ground substance is substantially unaltered. If it could be shown, for instance, that the segregation produced by centrifuging is a mass movement of the areas and zones of the protoplasm, and not simply a movement of granules through the ground substance, the entire organization of the egg would be altered, and there would be no reason why polarity should appear in the original direction rather than in any other direction.

I propose therefore to examine the thesis that the movements produced by centrifuging are purely granule movements, with the single exception of the nucleus or spindle. To do this it is necessary to compare minutely the structure of the egg both before and after centrifuging, using a fixed and determinate stage for such examination. Fortunately, the latter condition is a rela-

tively simple matter in the case of the egg of *Chætopterus*, because all ripe eggs reach such a stage in about twenty minutes after they are put in sea water, and undergo no further progressive development unless fertilized or otherwise suitably stimulated.

This stage may be described as follows (Fig. 4):

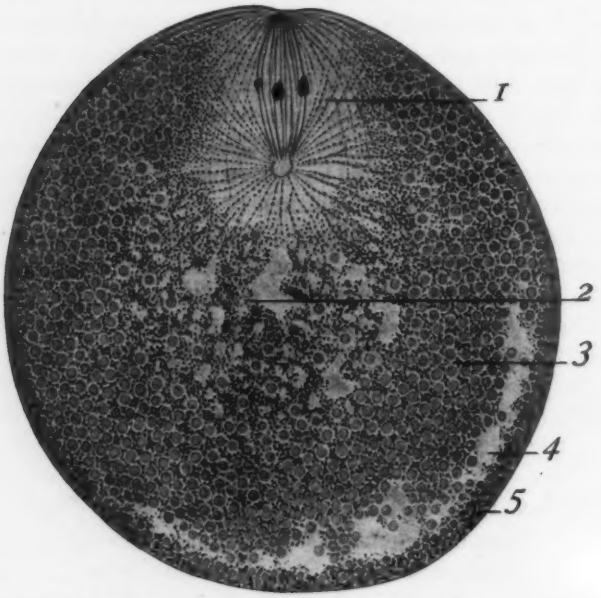

FIG. 4. Section of the egg of *Chætopterus* at the stage used for the experiments. See text for description.

The substance of the polarized egg shows the following areas and zones:

1. *The spindle area* lies at the animal pole and includes the spindle and the protoplasm in which the astral rays are developed. This area is predominantly basophile, but it may include a very few acidophile yolk granules. The astral rays may encroach slightly on (2) and (3).

2. *The central spongy area* lies near the center of the egg in the direct line of prolongation of the spindle. Its spongy appearance is due to paucity of granules and not to actual vacuoli-

zation of the ground substance. The basophile granules predominate and are arranged in a loose spongy network. This area is bounded by the spindle area on the animal pole side, and by the spherular zone (3) on the other sides. The boundary towards the spindle area is a little indefinite, fairly definite on the other sides. Its form is that of an indented sphere, the indentation being produced by the spindle area. Its position is about the same as that of the germinal vesicle before rupture.

Together, the spindle area and the central spongy area form an ovoidal mass, the small end of which reaches the surface at the animal pole, while the large end extends below the center of the egg.

The other zones of the egg are arranged concentrically to this mass, and are therefore open at the animal pole and thickest at the sides and vegetative end.

3. *The spherular zone* is so named because it contains the major part of the endoplasmic spherules or endoplasmic acidophile granules (yolk). In section, it appears as a very deep gourd-shaped crescent. The thin horns reach up above the level of the equatorial plate of the spindle.

4. *The intermediate spongy zone* lies between (4) and (5). In many eggs it is barely noticeable, in others it is very prominent. Like the central spongy zone, it is not a vacuolated portion of the ground substance, but it is characterized by paucity of granules, and so appears unstained in sections. It is a genuine structural feature as is proved by experiments, and also by the fact that it becomes more pronounced in eggs allowed to remain in the sea water (Fig. 5).

5. *The ectoplasmic zone* is the peripheral boundary of the egg, except that it is defective at the animal pole where the spindle area comes to the surface. It is characterized by the presence of spherules that differ in certain properties from those of the spherular zone (endoplasm), as described in detail in a previous paper.

In eggs allowed to stand in sea water without fertilization these areas become more strongly differentiated (Fig. 5), and the fact that the egg has a concentric as well as a polar organization stands out more strongly. The central spongy area now appears

to have a thick and dense wall in which the spherules of the spherular zone are especially aggregated. The intermediate spongy zone appears wider. The striking similarity of this con-

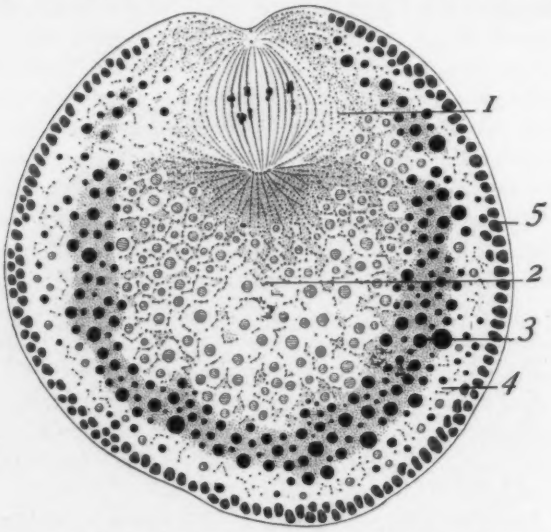

FIG. 5. Section of an unfertilized egg of *Chætopterus* that stood in sea water two hours and seven minutes before killing.

figuration to that produced by low centrifugal powers will be seen as the description proceeds.

We may now compare the pictures presented by eggs of this stage that have been centrifuged at different speeds: For this purpose we shall select first an egg that was centrifuged 1,150 revolutions in 31 seconds at a radius of 13 centimeters (Fig. 6). Comparison of a large number of eggs shows first that the position of the eggs in the centrifuge is a matter of chance and second that the segregation produced is essentially the same in whatever direction the centrifugal force may act with reference to the polar axis. We may therefore leave the question of direction of centrifugal force out of account. As already noted, the effect on the living egg is very striking: it appears stratified in the direction of action of the centrifugal force; at the central pole is a small gray cap, next to it a broad, clear or hyaline

band, and the distal hemisphere appears intensely yellow, and packed full of yolk-granules. The surface of apposition of the hyaline band and yellow hemisphere is not a plane surface, but decidedly convex towards the hyaline band.

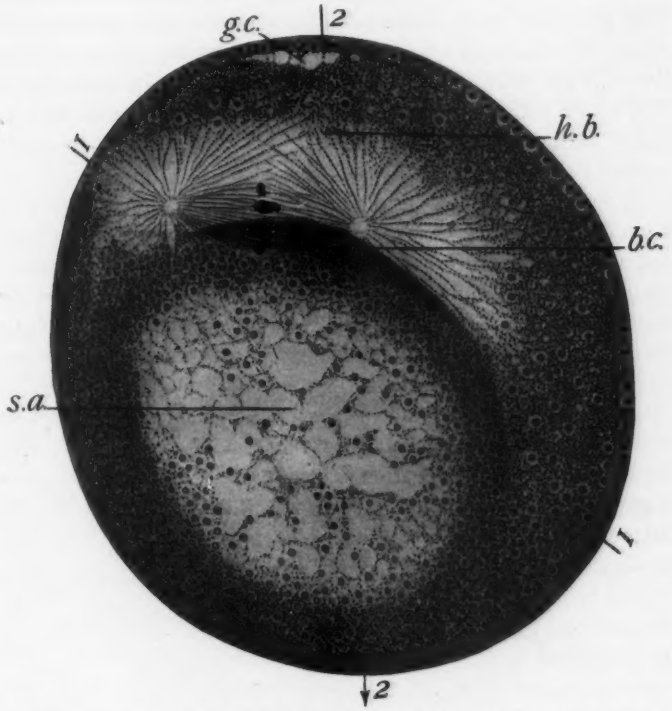

FIG. 6. Section of an egg of *Chætopterus* centrifuged 1,150 revolutions in 31 seconds at a radius of 13 cm. The force was not sufficient to produce a very full segregation of the material of the gray cap. *1–1*, primary axis of the egg; *2–2*, secondary axis. *b.c.*, basophile cap; *g.c.*, gray cap; *h.b.*, hyaline band; *s.a.*, spongy area; stained in thionin and orange *G*.

The gray cap is composed of fat globules, as is demonstrated by staining with osmic acid (Fig. 9); whether or not it includes the residual substance of the germinal vesicle as maintained in my former paper, I am not now prepared to say. In *Nereis*, in place of the gray cap, we have an aggregation of the large oil drops

characteristic of this egg. The hyaline band contains the small basophile granules, and the distal hemisphere the large acidophile granules (yolk-spheres). In sections stained in thionin and orange *G* the hyaline band is bright blue and very finely granular; the distal hemisphere is orange, owing to the affinity of the large yolk granules for this stain. But among the orange spherules are some basophile granules, and with the centrifugal power noted above, some orange spherules may be found in the basophile band. But with a sufficiently high power a practically complete separation of basophile and acidophile granules may be brought about.

Attention must now be directed to a definite configuration in the distal or yellow hemisphere, viz., a clear, spongy area which extends into the base of the clear band, and which is surrounded distally and laterally by acidophile granules. It is similar to the central spongy area of the normal control eggs with which it must be identified. It contains relatively few granules, which are arranged in a coarse meshwork. The central end of this area is occupied by an aggregation of basophile granules which are so much more densely packed than those in the clear band, with which the special aggregation is continuous, as to contrast strikingly with it in sections; particularly with low powers of the microscope. The form of this aggregation is lunate or biconvex in section, thick in the center and diminishing towards the ends.

The large acidophile granules tend in a distal direction and the smaller basophlle granules in a central direction. If there were no differences in resistance in the ground substance they would pass in straight lines. But the fact is that they do not do so, and this is *prima facie* evidence of differences in resistance of the ground substance in different areas:

The basophile cap in the central boundary of the spongy area is a clear demonstration of this principle: In the first place it is an aggregation of basophile granules; this is proved by the staining reaction, always basic. That the stain is in the granules and not in the ground substance is readily demonstrated in thin sections, and the granules are of the same size as the basic granules of the clear band. In the second place, since such basophile granules tend to move centrally, that is, towards the hyaline band,

under the influence of centrifugal force, this aggregation, which does not exist in the control eggs, must have come from the distal hemisphere. But as they lie at the central end of the spongy area, and are most numerous opposite the center of this area, and diminish towards the sides, it is obvious that they must have come from the interior of this area.

They are in fact a swarm of microsomes moving under the influence of centrifugal force, but delayed on the borderland of the

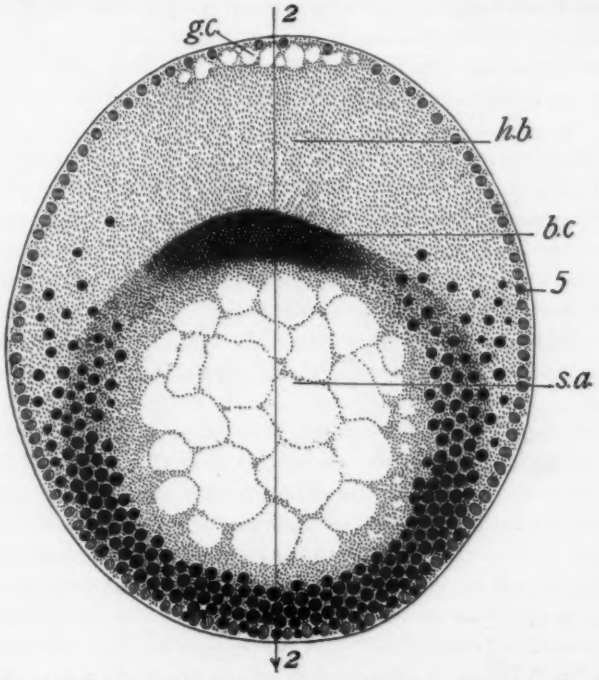

FIG. 7. Section of an egg of *Chætopterus* centrifuged 1,150 revolutions in 31 seconds at a radius of 13 cm. The primary axis of the egg is unknown in this case. *2–2*, secondary axis; *5*, ectoplasmic zone; *b.c.*, basophile cap; *g.c.*, gray cap; *h.b.*, hyaline band; *s.a.*, spongy area; stained in iron hæmatoxylin and orange *G*.

hyaline band (cf. Fig. 7), where they belong by virtue of their specific gravity, by the greater density (or other mode of resistance) of the wall of the spongy area.

This property of the wall of the spongy area also explains the

form of aggregation of the acidophile granules, which tend on this account to pass around and not into it. It also explains the more striking character of the spongy area in the centrifuged and the control eggs (cf. Figs. 6 and 4), because the granules within this area are free to segregate at its ends, but those without are prevented from entering.

Similarly, the persistence of the granules in the ectoplasmic zone indicates that this is another region of greater density of the ground substance. It requires a much greater centrifugal force to dislodge these granules than those lying within the egg, but when they are so dislodged they appear to be the heaviest class of granules within the egg, for they aggregrate at the distal pole (Fig. 9).

The following diagrams (Fig. 8) illustrate both the conception of the ground substance to which I have come as a result of the experiments, and the effect of the centrifugal force on the ground substance itself: The latter consists of four concentric layers, 2, 3, 4 and 5 (Figs. *A* and *C*); of which 5, the ectoplasmic stratum, is relatively dense, 4, the zone containing most of the acidophile granules of the normal egg is relatively fluid, 3, again, is a dense ring-shaped zone, surrounding 2, the central, more fluid spongy area.

The egg is an elastic sphere; it therefore undergoes elongation in the direction of the centrifugal force (Fig. 8, *B* and *D*); this is perfectly obvious immediately after centrifuging. The arrows in the figures to the left indicate the direction of the centrifugal force and the figure to the right in each case represents the result. Now observation shows that the spongy area of centrifuged eggs lies in the distal hemisphere, hence elongation of the ground substance must have taken place on the central side, owing presumably to the tension of the specifically very light substances (*e. g.*, fat globules) found here.

I have employed five or six different degrees of centrifugal force in examining this question and have made a considerable number of experiments with each. The configuration which we have just described varies somewhat with different degrees. With less force the same configuration is found down to 575 revolutions in 17 seconds with a radius of 13 cm. With higher forces

it tends gradually to disappear as is to be expected, for it is obvious that with a sufficient force the granules would be driven through or out of areas of greater density very readily and would segregate solely with reference to their specific gravity. With a

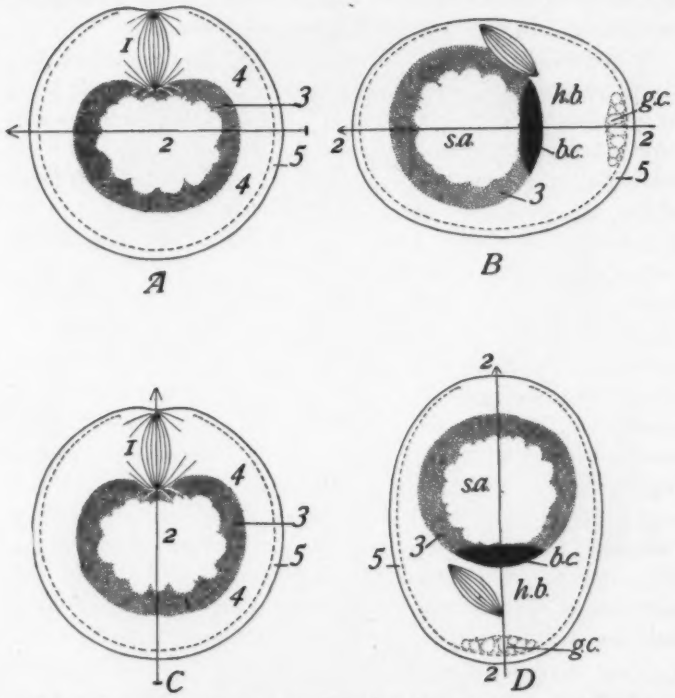

FIG. 8. Diagrams to illustrate structure of the ground substance of the egg of *Chætopterus* as revealed by centrifuging. *A* and *C*, controls: the arrow in each represents the direction of the centrifugal force. *B* and *D*, resultant conditions after centrifuging; *B* when the direction of the centrifugal force is as in *A*, *D* when the direction of the centrifugal force is as in *C*. *1*, spindle area; *2*, spongy area of control eggs; *2–2*, secondary axis of centrifuged eggs; *3*, dense ring-shaped zone in the ground substance; *4*, relatively fluid zone; *5*, ectoplasmic zone. The numbers have about the same significance as in Fig. 4. *b.c.*, basophile cap; *g.c.*, gray cap; *h.b.*, hyaline band; *s.a.*, spongy area of centrifuged eggs.

speed of 7,500 revolutions in one minute at a radius of about 6 centimeters, one can hardly observe any trace of the concentric arrangement (Fig. 9). Abundance of proof can be furnished for

these statements, but there would not be much value in multiplying instances by describing all of the intermediate conditions.

The same configuration is found in all stages of maturation and fertilization up to the first cleavage, at which time the cleavage naturally causes modification. Whether or not it reappears in each cell is a question that I have not examined.

It may be objected that the configuration shown could be produced as readily by mass movements of the protoplasmic areas as by individual granule movements. But such a conception would meet a fatal difficulty in the case of the central spongy area; the basophile cap in the central end (with reference to the centrifuge) of this area could not be produced in any other way than by a granule movement. Moreover, detailed examination of the movements in the spherular zone (Fig. 4, *3*) seem to me to be equally conclusive: If the movements produced by centrifugal force in this area were mass movements of the protoplasm as a whole the conformation of this zone should vary with the direction of the centrifugal force until it reached a final position of equilibrium, for the reason that it would fold in different ways around the central spongy area. The graded series of centrifugal forces employed may be regarded as showing stages in the segregation of the granules of this area. If the movements were mass movements the animal pole extensions of the spherular zone (Fig. 4) must wrap around the central spongy area where the centrifugal force acts at any appreciable angle from the egg-axis. But this is not the case; the proximal pole of the spongy area is never covered by such a fold. With the lowest speed, in directions at any appreciable angle to the egg-axis, one finds that the hyaline zone is dotted with granules of the spherular zone, evidently caught in migration; such granules are rarer with successively higher powers of the centrifuge, until they are practically absent. It is obvious that they pass individually across the hyaline zone.

Another conclusive proof of individual granule movements is shown in Fig. 9. The spindle is in a very high degree resistant to penetration, and it therefore often acts as a block to migrating granules, which pile up against it. The figure illustrates the blocking of fat granules which are for the most part accumu-

lated at the central end of the egg (upper end in the figure). Innumerable cases of this kind are found in the preparations.

Again, as already noted, the ectoplasmic granules tend to keep within the ectoplasmic zone with low powers of the centrifuge.

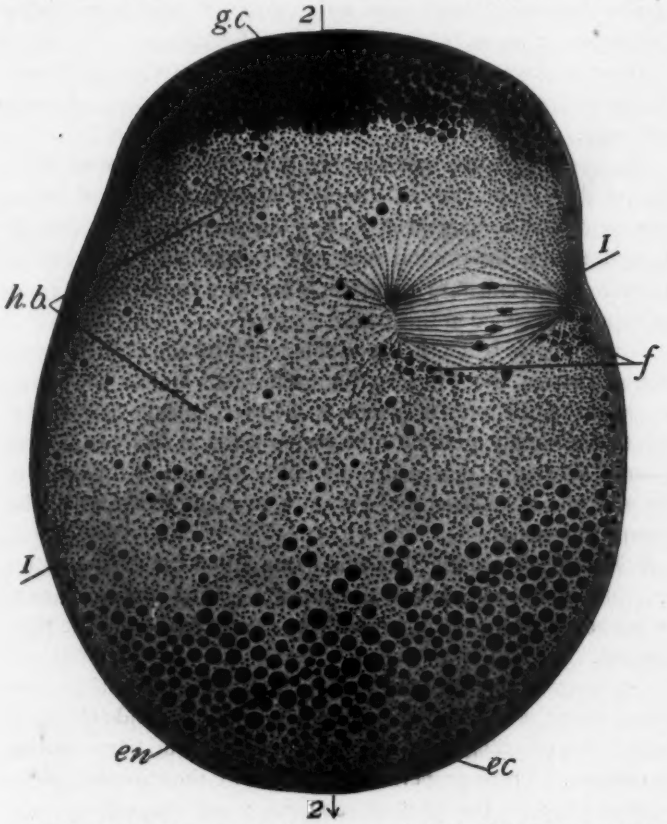

FIG. 9. Section of an egg of *Chaetopterus* centrifuged 7,800 revolutions in one minute at a radius of 6 cm. The egg was killed in Flemming's fluid, and the fat granules of the gray cap are stained black. The spindle blocks fat globules passing in a central direction (*f*). *1–1*, primary axis; *2–2*, secondary axis; *ec.*, ectoplasmic spherules; *en.*, endoplasmic spherules in the distal hemisphere; *f.*, fat globules blocked by the spindle, *g.c.*, gray cap; *h.b.*, hyaline band.

But with higher powers they pass to the distal pole of the egg. Nevertheless the ectoplasmic zone may be recognized without

the granules, after fixation with Flemming's fluid, as shown in Fig. 9.

The conclusion of this whole matter is that there is demonstrative evidence of individual movement of all classes of granules under centrifugal power, and no evidence of mass movements of protoplasmic areas. These movements reveal a definite form of concentric organization in the ground substance, which is only indistinctly revealed by the granule arrangement seen in the normal egg.

It does not require much argument to show that the organization of the ground substance is the primary factor that determines the concentric arrangement of the granules. The idea that the organization in the ground substance is secondarily produced by a given arrangement of granules is contrary to the entire series of results contained in this paper.

The granules reach their position and form the normal segregation pattern by "flowing" movements. Unless the entire argument of the present paper is incorrect, the flowing movements are simply granule movements within a firmly organized ground substance, determined by unknown factors involved in the relations of nucleus and granules to each other and to the ground substance.

It is incredible to me that vital organization can be bound up in a flux of all protoplasmic constituents. Vital processes must be bound up with some firm condition of aggregation in the protoplasm.

But how, it may be asked, are polarity and bilaterality connected with the concentric organization of the ground substance revealed by centrifuging? The answer to this question is not at all obvious. These properties are shown by the experiments to be properties of the ground substance; and the experiments further show, as illustrated in Fig. 8, that the ground substance is merely distorted, not essentially altered by centrifugal force, and this furnishes a physical basis for the persistence of polarity and bilaterality in the original directions. But their nature is not in the least revealed by the experiments. The results only furnish a partial answer to the old question, how life and organization can exist in a total flux of materials, by showing that the flux is only partial.

Morgan has made the suggestion (*Science*, August 28, 1908, p. 288) that formative substances other than the visible granules here referred to may possibly be present in the sea urchin's egg, and that such materials are not seriously disturbed by a centrifugal force sufficient to separate the visible substances of the egg; but that if these more fundamental substances are displaced, interference with the normal development would be expected. According to this conception vital organization would still be a function of equilibrium and interaction of diverse substances. The logical possibility of such a conception must be admitted, but it avoids none of the difficulties inherent in the usual hypothesis of organization, and seems to me to offer no possibility for explaining the polarity or bilaterality of parts of an ovum or the phenomena of regulation in general.

The existence of polarity and bilaterality in an optically homogeneous medium, and the persistence of both as to orientation under experimental conditions that seriously modify the quantitative relations of the oriented medium in different regions (as, for instance, when the yolk granules are packed closely into the small cell of the two-celled stage of *Chætopterus*) seem to me to argue for a molecular basis of the fundamental principle of vital organization.

Lehmann's observations on fluid crystals offer interesting analogies that are suggestive in the consideration of such fundamental biological problems. They show that the conception of crystallization as applied to vital organization does not meet with some of the difficulties formerly considered inherent in the analogy. Particularly his observations on the so-called myelin forms where innumerable fluid crystals determine the form and behavior of a single aggregate, suggest most interesting biological analogies, and aid to a certain extent in conceiving a doubly heteropolar condition of aggregation such as is presented by the animal egg.[1]

[1] It is not of course the author's intention in this communication to attempt to establish the validity of the analogy, but only to point out its possible application to the demonstrated organization of the ground substance of the cytoplasm. A good discussion of the entire analogy of crystallization to morphogenesis is contained in Przibram's article "Kristall-Analogien zur Entwicklungsmechanik der Organismen," *Archiv für Entw'mech.*, Bd. XXII., 1906.

The principle laid down here as to the molecular basis of cytoplasmic organization must apply not only to the stages considered but to all stages of development and to all parts of the organism.

The polarity of the egg and of living parts generally has been compared to magnetic polarity. Thus parts of a magnet exhibit polarity in the same direction as the original whole, and the north pole of the part is as true a north pole as the north pole of the whole magnet; the same is generally true of polarity in living things: a piece of *Hydra*, for instance, exhibits the same polarity as the entire animal of which it is a part. But push the comparison a little farther and the analogy appears to break down: a piece from the north end of a magnet has no more northness, so to speak, than a piece of the same size from the south end; on the other hand, an animal piece of an entire egg or an oral piece of an entire animal frequently has considerably more animal or oral properties than a piece from the vegetative or aboral end. The same difficulty of course arises if we use the analogy of crystallization. But it may be that it is due to secondary causes that modify the result without detriment to the validity of either analogy.

In conclusion a few words with reference to the so-called formative stuffs: So far as they are to be identified with the visible substances segregated by the centrifuge, it would appear to be indicated by the experiments that they can play no specific rôle in differentiation, because in centrifuged eggs they may occupy variable positions in the embryo. I believe, however, that the matter requires further investigation. It seems hardly credible that the strictly determinate distribution of these substances in normal ontogeny is meaningless, and that abnormal distribution is a matter of indifference. It is noteworthy that a large proportion of centrifuged eggs die before development has proceeded far, and that there is a distinct tendency towards abnormalities in the surviving eggs. It is natural to correlate these facts with unregulated abnormalities of distribution of granular materials. In any event a more careful study of the facts is needed.

It should be borne in mind that only the factors of polarity and bilaterality, the localizing factors in general, are shown to be functions of the ground substance. The respective rôles of

nucleus and granules are undetermined by these experiments; but their importance in the physiology of development is not open to question.

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A NEW NARCOMEDUSA FROM THE NORTH ATLANTIC.[1]

R. P. BIGELOW.

Late in the summer of 1899, the U. S. S. "Fish Hawk" of the Bureau of Fisheries made an expedition from Wood's Hole to the Gulf Stream. Upon her return I was handed a bottle containing a beautiful, clear, colorless medusa of considerable size, and was asked to identify it. The specimen was preserved in formalin and was in excellent condition. A short examination, however, was sufficient to prove it to be an unfamiliar form.

Fearing the deterioration of this unique specimen, I began at once in the Fisheries Laboratory at Woods Hole, as careful a study of its structure as could be made without destroying the specimen. Detailed, measured drawings were made, two of these being reproduced in the present article.

Unfortunately the otoliths were not visible, probably having been destroyed by the formalin, and the specimen appeared to be immature, as gonads were not distinctly marked. These circumstances made the identification difficult, and although the specimen appeared to belong to a new species, I hesitated to describe a new species from this single specimen. It was, therefore, put aside in the hope that another season might furnish material for a more complete diagnosis.

The material hoped for, however, has not appeared, and as my friend, Dr. A. G. Mayer, assures me that in all probability this is a new species of the genus Pegantha, I venture to publish the following description:

PEGANTHA CLARA, sp. nov.

General description. — Bell lenticular, doubly convex, 53 mm. wide, 20 mm. thick, exumbrella smooth, 28 equally spaced bell-lappets alternating with 28 tentacles; 14 of these tentacles are long, and range between 33 and 56 mm. in length, and they alter-

[1] Published by permission of Hon. George M. Bowers, U. S. Commissioner of Fisheries.

nate with 14 smaller tentacles ranging in length from 11 to 35 mm. (The specimen in hand has only 27 tentacles, one of the smaller series having failed to develop.) The insertions of the long set of tentacles are farther upward (above the margin) and inward

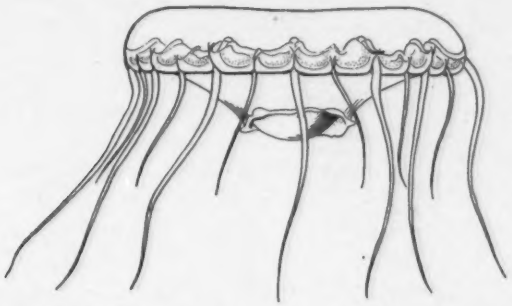

FIG. 1. *Pegantha clara*, side view. The tentacles are represented on one side only. Natural size.

(toward the center) than are the insertions of the short tentacles. The tentacles taper gradually to their pointed tips, and their entodermal cores are composed of a row of chordate cells.

The otolith-clubs appear to have been destroyed by the formalin in which the medusa is preserved; but there are 2–5, usually 3, long, slender, linear, somewhat tortuous, sensory tracts (otoporpæ) which extend from the bell-margin about one half the distance up the exumbrellar side of each lappet.

The velum is simple, annular, and provided with powerful cir-

FIG. 2. A portion of the margin of the subumbrella with the velum pressed out flat, and showing gastric pouches, festoon canal, and the transparent bases of the tentacles between them. Natural size.

cular muscles. The mouth is a simple annular opening at the thick lenticular center of the subumbrella.

At the margin of the stomach are 28 simple, unilobular saccules which project downward into the bell-cavity, one in each

antimere. These bag-like protrusions thus alternate with the radii of the tentacles, and they contain the genital organs, the specimen being a male. The marginal ring canal, or "festoon canal," is very wide. It extends down the sides of the peronial strand on either side of the insertion of each tentacle, and along the margin of each lappet. It is thus broken up into 28 loops, one in each antimere.

The gelatinous substance of the bell is hyaline. The tentacles, gonads, stomach, and festoon canal are milky — slightly brown in formalin.

The medusa upon which this description is based is probably immature; for one half of its tentacles are of small size, and belong apparently to a set that is in process of development.

Locality. — North Atlantic, U. S. S. "Fish Hawk" Station No. 7068, near the border of the Gulf Stream, off the southern coast of New England.